

## 2.2 Electrophoresis Module Assembly and Sample Loading

### Required materials:

- Clean and dry Mini-PROTEAN® Tetra cell tank
- Electrophoresis module (Electrode Assembly Module only for 1 or 2 gels; for 3 or 4 gels also use the Companion Running Module)
- Running buffer (700 ml for 2 gels; 1000 ml for 4 gels)
- Ready Gel® precast gels or hand-cast gels
- PowerPac™ Basic power supply

### 1. Assembly

**Note:** When running 2 gels only, use the Electrode Assembly (the one with the banana plugs), NOT the Companion Running Module (the one without the banana plugs). When running 4 gels, both the Electrode Assembly and the Companion Running Module must be used, for a total of 4 gels (2 gels per assembly).

- a. Set the clamping frame to the open position on a clean flat surface (see Figure 4a)
- b. Place the first gel sandwich or gel cassette (with the short plate facing inward) onto the gel supports; gel supports are molded into the bottom of the clamping frame assembly; there are two supports in each side of the assembly. Note that the gel will now rest at a 30° angle, tilting away from the center of the clamping frame.  
**Please use caution when placing the first gel, making sure that the clamping frame remains balanced and does not tip over.** Now, place the second gel on the other side of the clamping frame, again by resting the gel onto the supports. At this point there will be two gels resting at an angle, one on either side of the clamping frame, tilting away from the center of the frame (see Figure 4b).

**Note:** It is critical that gel cassettes are placed into the clamping frame with the short plate facing inward. Also, the clamping frame requires 2 gels to create a functioning assembly. If an odd number of gels (1 or 3) is being run, you must use the buffer dam (see Figure 4b).

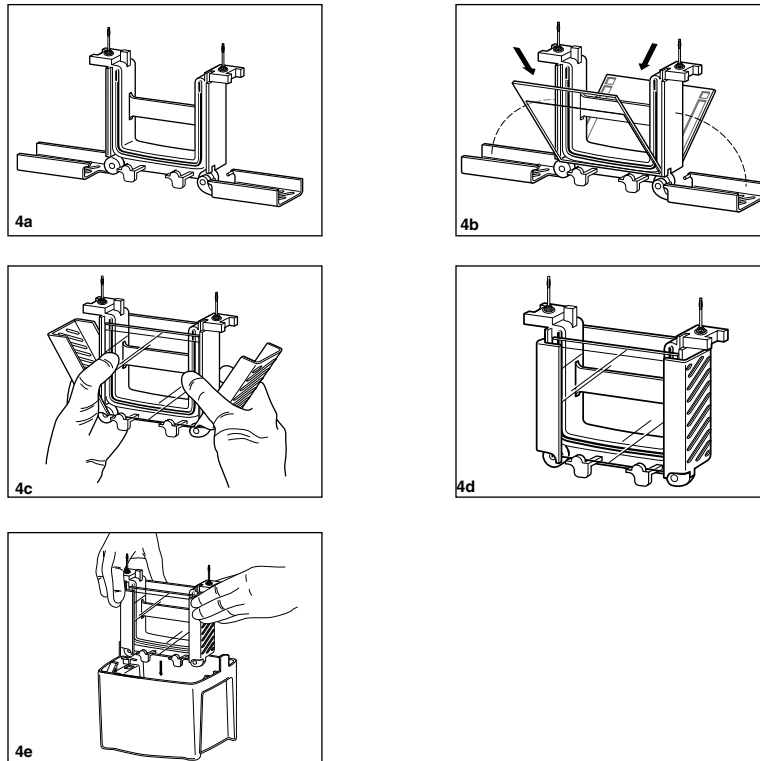
- c. Using one hand, gently pull both gels towards each other, making sure that they rest firmly and squarely against the green gaskets that are built into the clamping frame; make certain that the short plates sit just below the notch at the top of the green gasket.
- d. While gently squeezing the gel sandwiches or cassettes against the green gaskets with one hand (keeping constant pressure and both gels firmly held in place), slide the green arms of the clamping frame over the gels, locking them into place. Alternatively, you may choose to pick-up the entire assembly with both hands, making sure that the gels do not shift, and simultaneously sliding both arms of the clamping frame into place (see Figure 4c).

The arms of the clamping frame push the short plates of each gel cassette up against the notch in the green gasket, creating a leak-proof seal (check again to make certain that the short plates sit just below the notch at the top of the green gasket). At this point, the sample wells can be washed-out with running buffer, and sample can be loaded (Figure 4d).

**Note:** If running more than 2 gels, repeat steps 1a–d with the Companion Running Module.

**Important Note:** Do not attempt to lock the green arms of the clamping frame, without first ensuring that the gel cassettes are perfectly aligned and stabilized against the notches on the green gaskets of the module. To prevent the gels from shifting during the locking step, firmly and evenly grip them in place against the core of the module with one hand.

**CAUTION:** When running 1 or 2 gels only, **DO NOT** place the Companion Running Module in the tank. Doing so will cause excessive heat generation and prevent electrophoretic separation.



**Fig. 4. Assembling the Mini-PROTEAN Tetra Cell Electrophoresis Module.**

## **2. Sample Loading**

- a. Fill the assembly (upper chamber) with buffer to just under the edge of the outer gel plate.
- b. Load samples into each of the assemblies while they are sitting on a flat surface, outside of the tank.
- c. Load the samples into the wells with a Hamilton syringe or a pipet using gel loading tips.
- d. If using Bio-Rad's patented sample loading guide, place it between the two gels in the Electrode Assembly. Sample loading guides are available for 9, 10, 12, and 15 well formats.
- e. Use the Sample Loading Guide to locate the sample wells. Insert the Hamilton syringe or pipette tip into the slots of the guide and fill the corresponding wells.

**Note:** Load samples slowly to allow them to settle evenly on the bottom of the well. Be careful not to puncture the bottom of the well with the syringe needle or pipette.

**Note:** Samples may be loaded in the modules prior to placing the modules into the tank. Samples may also be loaded in the modules after the modules have been placed into the tank. Both methods will produce acceptable results. In both instances, the assembly (upper chamber) and the tank (lower chamber) should be filled with buffer as per 2.2.2a and 2.2.3d.

### 3. Placement of the electrode assemblies in the Mini-PROTEAN Tetra Tank.

**Note:** required total buffer volume, 700 ml for 2 gels; 1000ml for 4 gels.

The Mini-PROTEAN Tetra tank has two positions in which to place two assemblies: the Electrode Assembly (back position) and the Companion Running Module (front position).

- a. Begin by placing the tank on a flat surface, with the front of the tank facing you (the front of the tank is the face that has the 2-Gels and 4-Gels line markings); when oriented properly, the red marking on the top inside edge of the tank will be on your right, and the black marking on the top inside edge of the tank will be on your left.
- b. If running 2 gels only, you will be using just the Electrode Assembly, so place this assembly in the back position of the cell, making sure that the red (+) electrode jack matches the red marking on the top right inside edge of the tank.
- c. If running 4 gels, place the Electrode Assembly (banana plugs) in the back position (as detailed in 2.2.3b. above) and the Companion Running Module (no banana plugs) in the front position. Make sure that in both instances the red (+) electrode is matching with the red marking on the top inside right edge of the tank. Note that incorrect orientation will not permit proper placement of the lid.
- d. Fill the tank (lower chamber) with buffer to the indicated level (550 ml for 2 gels and 680 ml for 4 gels).

### 4. Mini-PROTEAN Tetra Tank Assembly

- a. Place the Lid on the Mini-PROTEAN Tetra Tank. Make sure to align the color coded banana plugs and jacks. The correct orientation is made by matching the jacks on the lid with the banana plugs on the electrode assembly. A stop on the lid prevents incorrect orientation. Note that the raised tabs on each side of the tank will now slide through the slots in the lid, guiding the lid to a proper close. At this point, firmly, yet gently, press down on the lid with your thumbs using even pressure, till the lid is securely and tightly positioned on the tank.

**CAUTION:** When running 1 or 2 gels only, **DO NOT** place the Companion Running Module in the tank. Doing so will cause excessive heat generation and will prevent electrophoretic separation.

### 5. Power Conditions

- a. Insert the electrical leads into a suitable power supply with the proper polarity.
- b. Apply power to the Mini-PROTEAN Tetra cell and begin electrophoresis; 200 V constant is recommended for SDS-PAGE and most native gel applications. The same voltage (200 V) is used for both 2 and 4 gels. The optimal voltage for your application may differ. Run time is approximately 35 minutes\* at 200 V for SDS-PAGE.

\* Electrophoresis time will vary between 35 and 45 minutes for Tris-HCl gels, depending on acrylamide percentage levels

## 6. Gel Removal

- a. After electrophoresis is complete, turn off the power supply and disconnect the electrical leads.
- b. Remove the tank lid and carefully lift out the electrode assemblies. Pour off and discard the running buffer.

**Note:** Always pour off the buffer before opening the arms of the assembly, to avoid spilling the buffer.

- c. Open the arms of the assembly and remove the gel cassettes.
- d. Remove the gels from the gel cassette by gently separating the two plates of the gel cassette.

**Note:** To remove the gel from a Ready Gel cassette, first slice the tape along the sides of the Ready Gel cassette where the inner glass plate meets the outer plastic plate.

- e. Remove the gel by floating it off the plate by inverting the gel and plate under fixative or transfer solution, agitating gently until the gel separates from the plate.
- f. Rinse the Mini-PROTEAN Tetra cell electrode assembly, clamping frame, and mini tank with distilled, deionized water after use.

## Section 3 Separation Theory and Optimization

### 3.1 Introduction

Polyacrylamide gel electrophoresis separates molecules in complex mixtures according to size and charge. During electrophoresis there is an intricate interaction of samples, gel matrix buffers, and electric current resulting in separate bands of individual molecules. Hence the variables that must be considered in electrophoresis are gel pore size, gel buffer systems, and the properties of the molecule of interest.

#### Gel Pore Size

Gel pores are created by the crosslinking of polyacrylamide with bis-acrylamide (bis) to create a network of pores. This structure allows the molecular sieving of molecules through the gel matrix. Gel pore size is a function of the acrylamide monomer concentration used (%T). By convention, polyacrylamide gels are characterized by %T, which is the weight percentage of the total monomer including the crosslinker. The %T gives an indication of the relative pore size of the gel. In general, pore size decreases with increasing %T.

%T is calculated using the following equation.

$$\%T = \frac{\text{g acrylamide} + \text{g crosslinker}}{\text{total volume (ml)}} \times 100\%$$

%C is the crosslinker:acrylamide monomer ratio of the monomer solution. %C is calculated using the following equation.

$$\%C = \frac{\text{g crosslinker}}{\text{g acrylamide} + \text{g crosslinker}} \times 100\%$$

2.67% C is traditionally used for most analytical gels.